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THE DEVELOPMENT OF BLASTOCLADIA STRANGULATA, N. SP.[1]

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(WITH PLATES XVIII–XX)

The genus *Blastocladia* was founded and incompletely described in 1876 by REINSCH (6) on the single species *B. Pringsheimii*, which for twenty years remained its sole representative. In 1896 THAXTER (9) rediscovered and made a careful study of the plant, which up to that time, apparently, had not been again observed. His studies cleared up several doubtful or unknown points in connection with its life-history and development, and led to the description of a new species, *B. ramosa*. Up to the present, so far as the writer is aware, no other species has been added to the genus. Aside from its peculiar characters, the genus is of particular interest because of its doubtful systematic position. Because of the resemblance of the resting spores to the deciduous conidia of certain *Pythium* species, THAXTER (9) placed the genus provisionally among the Pythiaceae. FISCHER (1) considers it with the genera which are doubtful or to be excluded from the Saprolegniaceae, while SCHRÖTER (7) includes it with the Leptomitaceae.

The species described in this paper fixes with considerable certainty, the writer believes, the true systematic position of the genus and is therefore described in detail.

This study was undertaken in the Botanical Laboratory of Cornell University under the supervision of Professor GEORGE F.

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[1] Contribution from the Department of Botany, Cornell University. No. 140.

ATKINSON, to whom I wish to express my thanks for his advice and kindly criticisms.

Material and methods

A single plant of this species was discovered growing on an aphid which had accidentally fallen into one of several water cultures prepared for the purpose of entrapping various Phycomycetes. The specific culture referred to was made from soil and decaying vegetation taken from the bottom of a small almost dry inland pond in the vicinity of Ithaca. When first observed the plant bore large numbers of resting sporangia, whose arrangement and bright orange color gave it a very beautiful appearance. After washing through several changes of sterile water, the plant was placed in a solid watch glass for further observation. On examination the following day it was found that a number of zoosporangia had developed, a few of which had already discharged the characteristic zoospores first described by THAXTER (9) for *B. Pringsheimii.*

Cultures were immediately started with aphids and other animal tissue, from which an abundance of material in all stages of development was secured. After making several unsuccessful attempts, a pure culture of the organism was obtained in the following manner: A few nearly mature zoosporangia were cut from a plant, carefully washed until practically free from contamination, and allowed to discharge their zoospores in sterile water. By means of a platinum loop the water containing the zoospores was spread over the surface of newly prepared slants of sweet corn agar. In a few days the small plants appeared as more or less isolated refractive specks on the surface of the agar, and were easily lifted out with a sterile needle and transferred to new tubes.

Material for sectioning was obtained in various stages of development from both water and agar cultures. It was soon found that the latter yielded just as good and more easily handled material than the former and it was therefore more frequently used. To secure the best results with the latter method, plants bearing nearly mature zoosporangia were transferred to the middle of a poured plate of either potato or sweet corn agar, preferably the latter, in a few drops of sterile water. After a few hours to one day large numbers of zoospores will have been discharged and can easily be spread over the plate by rocking the same or by the use of a sterile platinum loop. Thus distributed, the zoospores soon germinate and produce large numbers of usually simple plants bearing the reproductive organs. They commonly lie sufficiently close so that most of the agar may be cut out and fixed for microscopic study.

Three different killing solutions were used, which gave various results. These were medium chrom-acetic acid, Flemming's weak solution, and Gilson's fixer. After dehydration in the grades of alcohol or by evaporating down from 10 per cent glycerine, the material was cleared in cedar oil and imbedded in paraffin. Sections were cut 2–5 μ thick and stained on the slide.

The stains used were Flemming's triple stain with the orange G dissolved in clove oil, Heidenhain's iron-alum hematoxylin, and Gram's stain. The triple stain following Flemming's weak solution gave the best material for the study of the walls of the resting sporangia, papillae of dehiscence, and for fragmentation of the protoplasm to form zoospores; while Heidenhain's hematoxylin, when preceded by medium chrom-acetic acid, gave much the best material for the study of the protoplasm and nuclei. Gram's stain proved very good as a nuclear stain.

Description of the plant

The plant consists of a basal cell or cylinder whose lower extremity is attached to the substratum by a system of rhizoids, and supports above a dichotomously or umbellately branched system whose final branchlets terminate in one or more reproductive bodies (figs. 12, 58). At the points of origin of the branches, and occasionally elsewhere, there are more or less well marked constrictions of the mycelium. This character at once suggests a relationship to the members of the Leptomitaceae. The constrictions, however, are more abrupt and usually of less depth than those of that family. They mark off the plant into definite segments which are fairly constant in diameter throughout, although they occasionally have a tendency to enlarge slightly at one or both ends. This is especially noticeable in those branches which give rise to more than two branchlets. In this they resemble somewhat the branchlets of *Rhipidium americanum* Thaxter (**10**).

At the constricted points one finds pseudo-septa in various stages of development. These peculiar structures are unlike anything in the way of septa that I have seen described. In the older parts of the plant they reach their most perfect development, and then only incompletely separate the protoplasm of the adjacent segments. In a single well developed plant of some age, one may find the pseudo-septa in all stages of formation. They are first seen as separate processes or thickenings protruding inwardly from the wall at the constrictions. These processes increase in length, probably by accretion, until on meeting at the center of the cell, a fusion takes place and a definite central plate results.

Fig. 18, *a–d*, represents different stages of development of a septum, while figs. 19, 20, and 21 represent sections through such a stage as that shown in fig. 18, *a*. In fig. 19 the section is through one arm and the central plate, fig. 20 through two opposite radial arms and the central plate, while fig. 21 shows a section through the central plate alone. These pseudo-septa are much more highly differentiated than the "cellulin" rings which are present in *Gonapodya* and other Leptomitaceae. They permit a free interchange of the protoplasm, and only under conditions of injury to the hypha do they entirely close the lumen. Both the mycelial walls and the pseudo-septa fail to give any definite reaction for cellulose. After treatment with iodine and sulphuric acid, very rarely a slight trace of blue color is seen in the mycelial walls. The pseudo-septa become much swollen and take on a deep orange color, resembling in this respect the reaction secured by PRINGSHEIM (**5**) for the "cellulin granules."

In young actively growing plants the protoplasm is much vacuolated, granular, and contains, distributed throughout it, prominent nuclei containing deeply staining bodies (figs. 28, 32). Aside from the nuclei, there occur other deeply staining bodies which are more or less regular in form and of various sizes. They are very probably similar to those described by REINSCH (**6**) as independent, endogenously produced cells, which he was inclined to believe were the origin of the reproductive organs (see figs. 12, 28, 32). THAXTER (**9**) observed these same bodies in *B. Pringsheimii*, and saw no reason for assuming that they were other than masses of fatty protoplasm. Their true nature has not been determined, but, as will be shown later, they have at least one definite function in connection with zoospore formation. They are always present in more or less abundance, their extent of occurrence depending, to a degree at least, on growth conditions of the plant. In old plants, especially when the production of reproductive bodies has ceased, large groups of such bodies may be seen collected near the pseudo-septa, and frequently elsewhere in the mycelium.

Bodies somewhat similar in appearance are known to occur in the hyphae of members of the Saprolegniaceae. PRINGSHEIM (**5**) describes these at length and records a series of microchemical tests to determine their nature. According to his conclusions they are neither a proteid nor a carbohydrate substance, but rather waste products of metabolism.

Under favorable conditions of growth, a branchlet when terminated by a reproductive body may continue its growth by the production of a sub-branch (fig. 32). This sub-branch may be likewise terminated, sooner or later, and continued growth repeated as before. The length and rapidity of growth of these sub-branches determines whether the reproductive bodies shall occur at intervals or in a more or less compact head or group (figs. 6, 7, 12). This type of sympodial branching occurs in depauperate specimens of *Rhipidium americanum* (THAXTER **10**). It has also been noted for *Apodachlya.*

Under the best normal conditions for growth, the production of zoosporangia precedes that of resting sporangia. In pure cultures this order is easily reversed by properly manipulating external conditions. In a frequently refreshed culture, zoosporangia alone are at first produced, while on the other hand plants in a culture started and maintained in a small amount of water usually give rise to resting sporangia only.

The extent to which branching may proceed before the production of reproductive organs varies greatly. This may continue until a well formed almost hemispherical tuft is produced, or on the other hand zoosporangia may develop soon after the germination of the zoospore on the terminal end of the more or less elongated basal cell. This condition occurs particularly on young plants started on agar and subsequently transferred to water. If sufficient moisture is present the same thing may take place directly on the agar (figs. 4, 5). Zoosporangia may be produced singly or in chains (fig. 59).

The growth of the plant is rapid when the best external conditions are offered. Observations made relative to this point showed that in one case hyphae which were just emerging from the body of an aphid at 10:30 A.M. had produced at 5:30 P.M. of the same day mature zoosporangia, some of which were discharging zoospores. The size of individual plants varies greatly with the purity of the cultures and the nature and amount of nutrient material at hand.

Development of zoosporangia

The zoosporangia are broadly oval to almost spherical, rarely elliptical, smooth, hyaline, and fairly constant in form and size. They may originate terminally or subterminally on the branchlets. In the former case the first indication of such a development is a slight swelling of the hyphal end accompanied by a well marked change in the protoplasm (fig. 11, *a–c*). Before reaching its normal size, the zoosporangium becomes cut off from the hypha by a septum, and papillae of dehiscence begin to appear. When produced subterminally, the mature zoosporangium may be borne on a more or less elongated branchlet, as previously noted, which originated from the parent branch directly below another reproductive body (fig. 6), may be sessile (fig. 12, *b*), or even develop as swollen segments cut off by septa (fig. 12, *a*). The latter method when continued produces a chain of zoosporangia (fig. 59). As a result of cutting off the ends of fertile branches, zoosporangia may bud out from the remaining parts in various places. In certain instances the contents of the injured branches give rise to zoospores without any modification in form.

The zoosporangia of *B. Pringsheimii*, after discharging their zoospores, drop from the plant, leaving numerous scars. This was noted by THAXTER (9) and also by PETERSEN (4). I have never observed this phenomenon to take place in *B. strangulata*. Old empty zoosporangia may be seen still attached to the plants weeks after they have ceased to produce reproductive organs.

As growth of the zoosporangium proceeds, there is a noticeable condensation of the protoplasm in the center, around which can be seen a number of indistinct vacuoles of irregular shape. There is little apparent change from this condition until the zoosporangium has reached its maximum size. The contents then become coarsely granular and no vacuoles are apparent. This stage may persist for some time if conditions for further development become poor. In fact, it is in this stage that zoosporangia rest at times for days. Suddenly the coarse granular character changes to one with fine evenly distributed granules, and the whole contents assume a much lighter appearance. After 15–30 minutes one can discern the formation of areas surrounded by faint granular but irregular lines. These become rapidly more prominent, and in a few minutes a slight movement can be detected within the zoosporangium. The areas represent the zoospores and their discharge is about to take place (fig. 8). The papillae of dehiscence, sometimes numbering as many as eight, become more and more extended and refractive until one or more finally break open, permitting the zoospores to escape. They pass out in single file, at first rather rapidly, then more slowly as the pressure within the zoosporangium becomes lessened (fig. 56). Being of a plastic nature, they squeeze through the opening, arriving at the outside irregular in form, and very commonly with their cilium held in the opening by the next emerging zoospore. After a few seconds they move slowly away, assuming their normal form.

The zoospores are oval to elliptical, not infrequently slightly ovate, in which case the narrower end is the anterior one. The number of cilia varies from one to three, and they are attached at the posterior end. From a large number of careful examinations of both living and stained preparations of zoospores, I assume that the uniciliated condition is the typical one, as it occurs much more frequently than the other two types. The triciliated zoospore is rarely seen, while the biciliated form is common. The zoospores of *B. Pringsheimii* possess, according to THAXTER (9), one or two cilia. He considered the latter number the typical one, while PETERSEN (4) has described the zoospores as uniciliate.

The zoospore contains a large subtriangular centrally located body which resembles a large nucleus (fig. 23). THAXTER observed this body and described it as follows: "The nucleus is very large and subtriangular in outline, its base connected with that of the cilia by a fine strand of protoplasm." Fig. 24 shows very distinctly the connection of the cilium with the base of the large central body. The zoospore was killed with a 1 per cent solution of osmic acid and stained with an alcoholic solution of Magdala red. In the process the outer portion of the zoospore broke away, leaving the cilium still attached to the central body as represented. There can be observed in properly killed and frequently in living zoospores a more or less hyaline globule situated at the base of the central body, which contains a highly refractive granule. This body is undoubtedly the nucleus of the zoospore and will be more fully discussed later. The zoospore also contains groups of large and small granules, evidently of a fatty nature, which are principally located in front and to the rear of the so-called central body.

In movement the zoospores proceed in a more or less direct course, with a slight swaying of the body, and at times accompanied by a slow rotation on the longitudinal axis. If supplied with sufficient oxygen they may continue to swim for a number of hours, but when mounted on a slide under a cover glass, where the oxygen supply is small, they soon cease movement and germinate.

Fig. 28 shows a section of a young reproductive body, presumably a young sporangium, stained with iron-alum hematoxylin. The protoplasm is granular, vacuolated, and contains distributed throughout it prominent nuclei and large and small deeply staining bodies to which reference has already been made. The number of nuclei is at first small, and there is apparently no marked passage of the nuclei from the adjacent portions of the mycelium such as occurs in the developing sporangia and sexual organs of many other Phycomycetes. The nuclei in the upper half of the sporangium are in various stages of division. This condition may be found in young rapidly growing hyphae and principally at the growing point. As growth proceeds the number of nuclei rapidly increases, until 40–70 are produced, usually about 60 in average-sized sporangia. About the time the zoosporangium reaches its full size the nuclei arrange themselves about the periphery. A large number of the sections show this condition, which seems to indicate that the zoosporangia rest in this stage. This condition probably agrees with that described above for the living specimen in which the protoplasm is coarsely granular.

Fig. 29 represents a section of a zoosporangium which is entering the stage of zoospore formation. The large nuclei have become distributed throughout the more coarsely granular protoplasm. The number and size of the deeply staining bodies has increased, while some of them show a vacuolate condition. On some of the nuclei can be seen deeply staining masses of small size. Other nuclei are associating themselves, more or less closely, with some of the larger masses.

This condition is carried still farther in fig. 30. In a number of cases the nuclei are more or less imbedded in the deeply stained bodies, in others they are still free from them. These stages very probably correspond to the more or less homogeneous stage of the living zoosporangium which just precedes the differentiation of the zoospores. Fig. 30 also shows the beginning of segmentation of the protoplasm. It proceeds from the periphery inward in a more or less radial direction, much as described by HARPER (**2**) for *Synchytrium decipiens*. The lines of division are first recognized as rows of granules, at first more or less indefinite, but which become more and more apparent until they are seen entirely to outline the spore mass.

Fig. 31 represents a part of a section of a sporangium in which segmentation is almost complete. The limiting surfaces of the spore masses in a number of cases have separated. Apparently contraction has taken place, which would indicate that the mature zoospores occupy less space than the original masses of protoplasm from which they are formed. HARPER (**2**) observed that shrinkage of the protoplasm takes place in the early stages of cleavage in *Synchytrium decipiens*, and suggested a loss of water as the cause. He also observed the open spaces formed by the separation of the segmented masses.

It will be observed (fig. 31) that the nuclei with their associated material have assumed a more regular form. The nucleus itself is drawn out to a point which, in some cases, extends to the very limits of the spore. No indications of cilia have been observed at this stage, but they can be seen occasionally in a later stage, that is, at the time of discharge of the zoospores. The condition of the nucleus described strongly suggests that the cilia have their origin through its direct influence.

Segmentation usually results in the formation of uninucleate zoospores. Occasionally, however, one may find binucleate zoospores with the nuclei in the same or different central bodies, or what I shall hereafter call food masses or bodies. Fig. 34 shows normal zoospores ready to escape from the zoosporangium. Fig. 35 shows the two types of binucleate zoospores of the same age as those in fig. 34.

In preparation for germination the zoospore comes to rest, takes on a spherical form, and gradually absorbs its cilium, which in the process commonly becomes enlarged at the end (fig. 25). The large reserve food body disappears and a large number of variously sized granules take its place. In the course of 10–20 minutes the germ tube makes its appearance and grows rapidly, forming the basis for the subsequently developed rhizoid system (figs. 26, 27, 2, *a–c*). The body of the zoospore forms the basal cell of the plant.

Fig. 36 represents a zoospore stained with iron-alum hematoxylin, preparing to germinate. As described above, the reserve food mass has apparently broken up into a number of deeply staining granules. As the germ tube elongates, the nucleus increases in size (fig. 38) and finally divides to form two (fig. 39). Accompanying the rapid growth of the young plant the protoplasm becomes more and more vacuolated and finally granular (fig. 40). Stained preparations of germinating zoospores beyond the four-nucleate stage were not obtained.

Just what the nature of the so-called reserve food bodies is has not been determined. Fig. 41 shows a zoospore killed with iodine solution. The nucleus and some granules show distinctly, while the large food body in most cases is invisible. Fig. 42, killed with weak Flemming solution, reveals that body clearly, and also the blackened condition of the granules, which indicates their fatty nature.

Papillae of dehiscence occur on the zoosporangia of many Phycomycetes. In most cases they have been described as small swollen areas in the sporangial wall, or tips of exit tubes which become gelatinized and allow the emission of the zoospores to take place. An interesting condition is found in *Rhipidium americanum*. The zoosporangium possesses a double wall; the outer forms a cap over the papillae, which, on the discharge of the zoospores, becomes lifted up by the protruding inner wall; the latter forms a cylinder or vesicle which incloses the discharging zoospores; it ruptures immediately, setting them free (THAXTER **10**). A similar condition prevails for *Sapromyces*.

So far as I have been able to learn, the structure and behavior of the papillae of *B. strangulata* differ from anything yet described. In the living sporangium the papilla possesses what appears to be an outer highly refractive hyaline convex cap, with a less refractive area between it and the protoplasm of the sporangium. The external part becomes more and more convex as gelatinization proceeds (fig. 8). Just before the disappearance of the outer part it loses its high refractive power to some extent, and has the semblance of glycerine. Suddenly the thin ungelatinized portion of the wall breaks, and becomes forced out, leaving a ragged rim, many times, about the opening. The adjacent gelatinized part is immediately dissolved in the surrounding water, and to all appearances the exit pore is open. In a few seconds the zoospores begin to escape. Under what seem to be normal conditions, a vesicle is formed which incloses at least a part of the zoospores on their discharge. This vesicle soon breaks and the zoospores are set free (fig. 13). Frequently no such vesicle can be seen, and in such cases the zoospores escape as shown in fig. 14. The formation of such an inclosing membrane immediately suggested a double wall to the zoosporangium, and also that it was the inner of the two which either protruded as in *Rhipidium americanum*, or that by its gelatinization it was enabled to stretch out in the form of a thin sac.

On the examination of sections it was found that neither assumption was correct. Fig. 9 shows a section through an immature papilla and the adjacent wall of the zoosporangium. It is very evident that the wall is single, but that there are two distinct parts to the gelatinized plug of the papilla. The plug has a strong affinity for stain, especially safranin. The ungelatinized part of the wall is seen as a thin unstained layer extending over the convex plug. The wall immediately surrounding the papilla is thickened so as to form a sort of collar. This is clearly seen in empty sporangia. Fig. 10 shows the two parts of the plug separated as a result of cutting the section. It will be seen that the inner portion bears a close relation to the protoplasm. Such a section is common. In those sections which show a contraction of the protoplasm from the wall of the zoosporangium, almost invariably it is found to adhere closely to the inner part of the plug, whether that remains in place or not. It is this part of the plug that has the less refractive power in the living state and that on stretching out forms the vesicle referred to above. Very probably gelatinization is brought about by the action of an enzyme secreted by the protoplasm in the region about the papilla, which may account for the close relation between the two just described. Apparently the outer part of the plug becomes more thoroughly gelatinized than the inner, while the outer thin unstained part of the wall over the papilla is little or not at all affected.

This condition may be explained, it seems, by assuming that the wall of the zoosporangium is made up of lamellae which differ slightly in composition and which are differently affected by the gelatinizing agent. This assumption is strengthened by the fact that in a few sections the condition illustrated in fig. 9 was observed, that is, the line separating the two portions of the gelatinized plug extended slightly into the sporangial wall.

Development of resting sporangia

The resting sporangia agree in general to similar bodies described by REINSCH (6) and THAXTER (9) for *B. Pringsheimii*, and by THAXTER (9) for *B. ramosa*. In *B. Pringsheimii* they are called resting spores by THAXTER, and are considered as doubtful oospores by REINSCH. They are indicative of the older condition of the plant, and when mature are deciduous. According to THAXTER, the mature resting spore is surrounded by two walls, an outer, thin and smooth, and an inner, thick and apparently perforated or pitted. He did not study a section of the wall, hence was not able to determine the structure definitely. They possess several large oil globules, are oval to pyriform, and vary in form almost as much as the zoosporangia.

In *B. ramosa* the resting sporangia, or spores, are "bluntly rounded, gradually narrower toward a truncate base, and about 30×11 μ." In this species the resting spores are less variable in form than those of *B. Pringsheimii*, but vary somewhat in size. The walls are very little thicker than those of the sporangia. Resting spores of neither species were seen to discharge zoospores or to germinate.

The resting sporangia of *B. strangulata*, for such they are, as will be shown later, are very constant in form and vary only slightly in size. They are ovate in form, with the narrower basal end truncate (fig. 15). As previously noted, they occur at almost any age of the plant, depending on the conditions of growth, and remain attached at maturity. They have their origin, in general, in the same manner as the zoosporangia, and in the younger stages cannot be distinguished from them. The wall begins to thicken early, and this, together with the absence of papillae, indicates that resting sporangia are developing. When they are mature they possess three walls, an outer and an inner, thin, smooth, and hyaline, and a middle, thick, perforated, and orange colored. The peculiar perforated nature of the middle layer can easily be made out in sections of mature resting sporangia. The pores are conical in shape, with the broad end outward. The greatest diameter is 0.8 μ and the least 0.3 μ. Fig. 16 shows a surface view, while fig. 17 is a diagrammatic representation of a cross-section, showing the pores and the inner and outer walls. The pores are more or less regularly arranged in rows, as seen in fig. 15.

The mature resting sporangia contain a number of oil globules which are not strikingly noticeable as they are in many other resting bodies of a similar nature. In describing these bodies for *B. Pringsheimii*, PETERSEN (4) says: "Peculiar pointed or rounded cylindrical resting spores with a flat base and remarkably porous walls, without much content, appear in place of the zoosporangia."

In the young stages the protoplasm has much the same appearance as that of the young sporangia. Fig. 32 represents a section of a developing resting sporangium in which the nuclei are arranged about the periphery. The central mass of granular protoplasm, containing several reserve food bodies, is surrounded by prominent vacuoles. In the mature sporangium the protoplasm forms a definite regularly arranged network in which the nuclei are distributed (fig. 33). There are present, also, deeply staining masses, more or less irregular in shape, which probably represent the fusion of several reserve food bodies.

On germination the contents of the resting sporangia escape in the form of zoospores not unlike those formed in the zoosporangia. According to all observations and tests made, it is necessary that the resting sporangia pass through a period of rest before germination. I have not found any to germinate that were not at least one month old. This applies to cultures developed both in water and on agar. On the transference of the sporangia to fresh water there is no apparent change for some time. After 18–20 hours the two outer walls become cracked open, due very probably to the absorption of water. Sometimes they crack in several places, bringing to view the inner hyaline wall which bears one or more papillae of dehiscence. In a short time the normal discharge of zoospores takes place (fig. 22).

Nuclear division

The resting nuclei are usually spherical in form and contain a large deeply staining body which I assume to be a chromatic nucleolus. Surrounding this body is a fine granular cytoplasm which can be seen forming an irregular network (fig. 43). They vary in size from very small, almost invisible dots, to those with a diameter of 6–7 μ. The smaller are found in the actively growing parts where nuclear division more commonly takes place. The mode of division is rather unusual and suggests a form of amitosis. The first indication of such a nuclear division is a change of the chromatin mass from the more or less normal spherical to an elongated form (figs. 48, 52). A transverse line of division is next seen (fig. 47). The two parts then round up (figs. 45, 53), separate (figs. 49, 51), and appear as two large nucleoli. A wall is finally laid down between the daughter chromatin masses, and the two nuclei result (figs. 44, 55). Frequently nuclei may contain three of these bodies (fig. 50). Evidently one of the two daughter masses of the first division divided before any nuclear wall separated them.

In dividing nuclei stained with iron-alum hematoxylin, there is a faintly staining homogeneous substance connecting the separating chromatin masses, which suggests some sort of a spindle. Two explanations suggest themselves: (1) that we are dealing with direct nuclear division and that the faintly staining substance is the cytoplasm contracted about the dividing chromatin masses; and (2) that division is indirect and that the large chromatin mass represents a single chromosome.

It seems unusual, if not improbable, that such a highly differentiated plant in so many respects should possess only a direct method of nuclear division. The fact, however, that no sexual organs are known for any of the species of the genus may have some bearing on the question. HUMPHREY (3) found a very similar nuclear division to take place in the hyphae of *Achlya apiculata*. From all observations yet made, I am inclined to hold to the view that we are dealing with a peculiar type of mitotic division. Further studies concerning the question are contemplated.

Since the species appears to be a new one, I add here a description giving the more important characters as observed by the writer.

Blastocladia strangulata, nov. sp.—Main axis oval to cylindrical, divided at the base into a number of rhizoidal divisions; above giving rise to a one to several times dichotomously or umbellately branched system whose ultimate branchlets produce terminally or subterminally zoosporangia and resting sporangia; definite constrictions and perforated pseudo-septa at the points of branching. Zoosporangia, oval to nearly spherical (50–63×40–52 μ), possess several papillae of dehiscence, and produce a comparatively small number of rather large zoospores. Zoospores 12×8 μ, with one to three cilia, usually one. Resting sporangia ovate to nearly oval, with a truncate base, 45×35 μ; the wall consists of three layers, the middle thick, perforated, and orange colored; on germination giving rise to zoospores. Whole plant 200–2000 μ high, its main axis 40–100 μ in diameter.

Found but once, and on an aphid in a water culture made from soil taken from the bottom of an almost dry inland pond near Ithaca, N.Y.[2]

Axi primario ovato ad cylindricum, basi divisionibus rhizomatoideis numerosis, sursum copiose dichotome v. subdichotome ramoso; ramosis intervallis constrictis; pseudo-septis perforatis intra ramulorum basim formatis; zoosporangiis ovalibus ad sphaeroidea, 50–63×40–52 μ, papillas dehiscentes paucas ferentibus; zoosporis ovalibus, 12×8 μ, cilio plerumque simplici ornatis; sporangiis perdurantibus, rotundatis, basim versus graditim angustioribus et truncatis, 45×35 μ, zoosporis foventibus.

Hab. Ad aphid in aqua, Ithaca, N.Y.

Summary

1. The plant resembles in general the other species of the genus. Its mycelium is definitely constricted, which fact, it seems, definitely places the genus in the family Leptomitaceae.

2. It possesses peculiar perforated pseudo-septa which are formed at the constrictions, and which in a way are comparable to the "cellulin rings" of other members of the Leptomitaceae.

3. Zoosporangia are provided with a number of papillae of dehiscence distributed over the surface, which are formed as the result of the gelatinization of small circular areas of the wall. The resulting plug is made up of two distinct parts, the inner of which forms a vesicle into which the zoospores escape at the time of their discharge.

4. The zoospores possess a large centrally located subtriangular mass of apparently some reserve food substance, probably proteid in nature, at whose base is located the nucleus. They are typically uniciliated, with the cilium in direct relation to the nucleus.

5. Resting sporangia possess a three-layered wall; the outer and inner layers thin and hyaline; and the middle thick, perforated, and orange colored. After a period of rest of several weeks, germination takes place by the formation of zoospores.

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[2] During May and June 1911 this species appeared several times in water cultures made from two garden soils one-fourth mile apart near Urbana, Ill. As it was found in soil collections made at different times and parts of the gardens, it was evidently not rare in those particular locations.

6. On germination the zoospore produces a germ tube which forms the basis of the rhizoid system, while the body of the spore becomes the basal cell of the plant.

7. Nuclear division is somewhat unusual, apparently, and reminds one of amitosis. It seems to the writer, however, that it is more probably a form of mitotic division dealing with a single large chromosome.

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EXPLANATION OF PLATES XVIII–XX

Fig. 1.—Biciliated zoospore.

Fig. 2.—Different stages in the germination of the zoospore.

Fig. 3.—Young plant with basal cell showing rhizoids and two branches which are beginning to branch dichotomously.

Fig. 4.—Young plant started on potato agar and subsequently transferred to water where sporangial development took place.

Fig. 5.—Plant similar to the one shown in fig. 4, with an empty sporangium and another almost mature below it.

Fig. 6.—A branch with sympodial arrangement of resting sporangia.

Fig. 7.—Resting sporangia more closely arranged on the branchlet, a more frequent condition in old cultures.

FIG. 8.—A mature sporangium showing zoospores differentiated and two papillae of dehiscence.

FIG. 9.—A stained section of a papilla of dehiscence.

FIG. 10.—Another section of a papilla of dehiscence, showing the protoplasm and attached inner portion of the plug drawn away from the wall.

FIG. 11.—Early stages in the development of zoosporangia.

FIG. 12.—A mature plant, showing the rhizoid system, manner of branching, and arrangement of reproductive bodies.

FIG. 13.—A mature sporangium discharging its zoospores into a thin vesicle which soon ruptures.

FIG. 14.—A mature zoosporangium discharging its zoospores without the formation of a vesicle.

FIG. 15.—Resting sporangium, showing the relative position of the pores in the much thickened wall.

FIG. 16.—An enlarged portion of the surface of a resting sporangium.

FIG. 17.—A diagrammatic representation of a microscopic section of the wall of a resting sporangium.

FIG. 18.—Different stages in the development of the pseudo-septa located at definite constrictions in the hyphae.

FIGS. 19–21.—Microscopic sections through such a septum as shown in fig. 18, *a*, giving the various appearances that result.

FIG. 22.—Germinating resting sporangium: outer walls cracked open, exposing the inner thin wall bearing papillae of dehiscence.

FIG. 23.—A zoospore showing the nucleus with its accompanying mass of reserve food material, and two cilia.

FIG. 24.—Zoospore killed with a 1 per cent solution of osmic acid and stained with Magdala red; the outer portion of the zoospore broke away, leaving the reserve material and nucleus with the single cilium attached.

FIG. 25.—Zoospore coming to rest preparatory to germination; contraction of the cilium taking place and contents becoming granular.

FIGS. 26, 27.—Early stages in germination of the zoospore: from living specimens.

FIG. 28.—Section of a young zoosporangium, showing nuclei in division and also several large deeply staining food bodies.

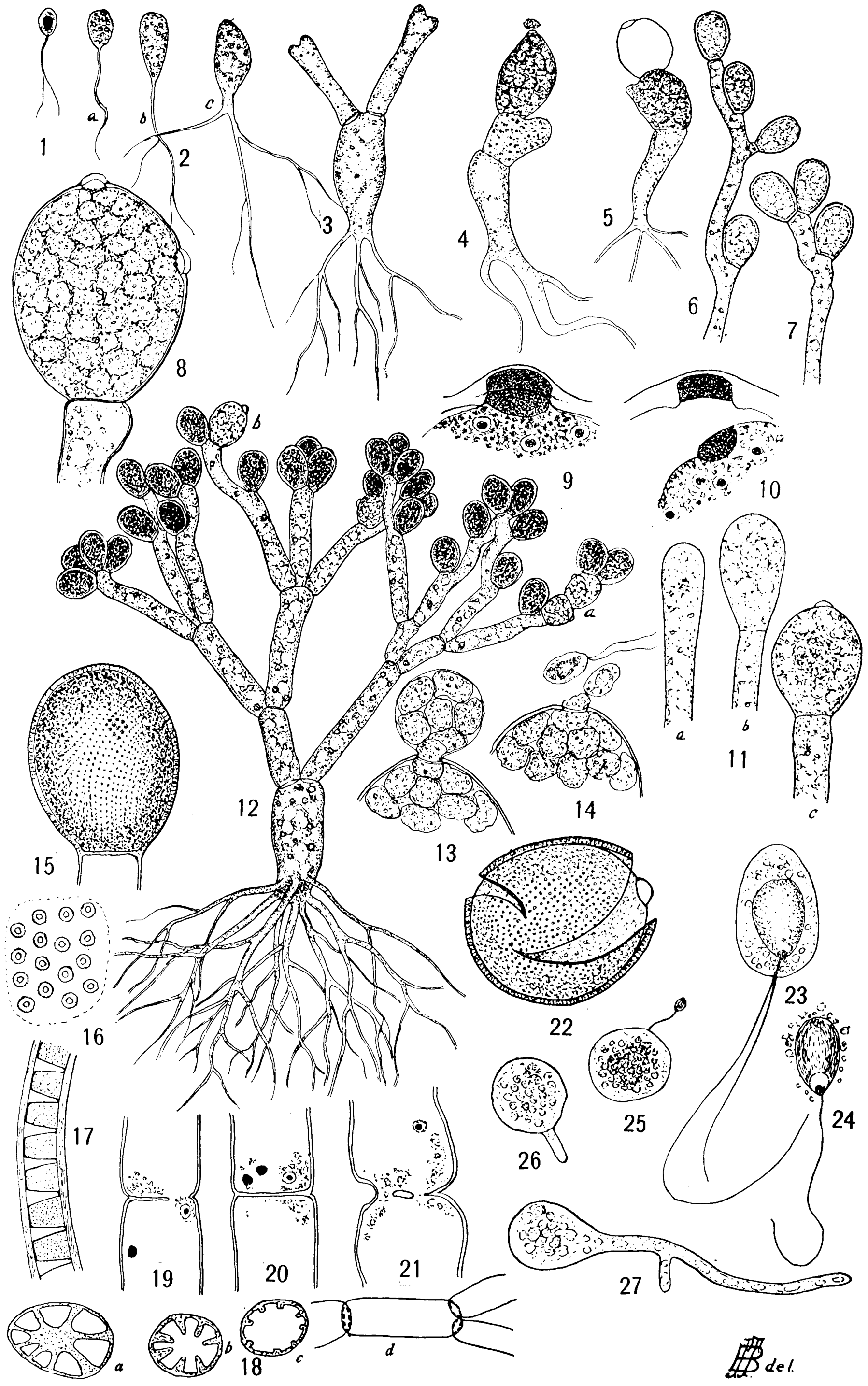
FIG. 29.—Section of a zoosporangium at the time of early nuclear association with the reserve food material preparatory to zoospore formation.

FIG. 30.—Later stage than that shown in fig. 29: many nuclei more or less imbedded in the reserve food material; lines of segmentation appearing at the periphery.

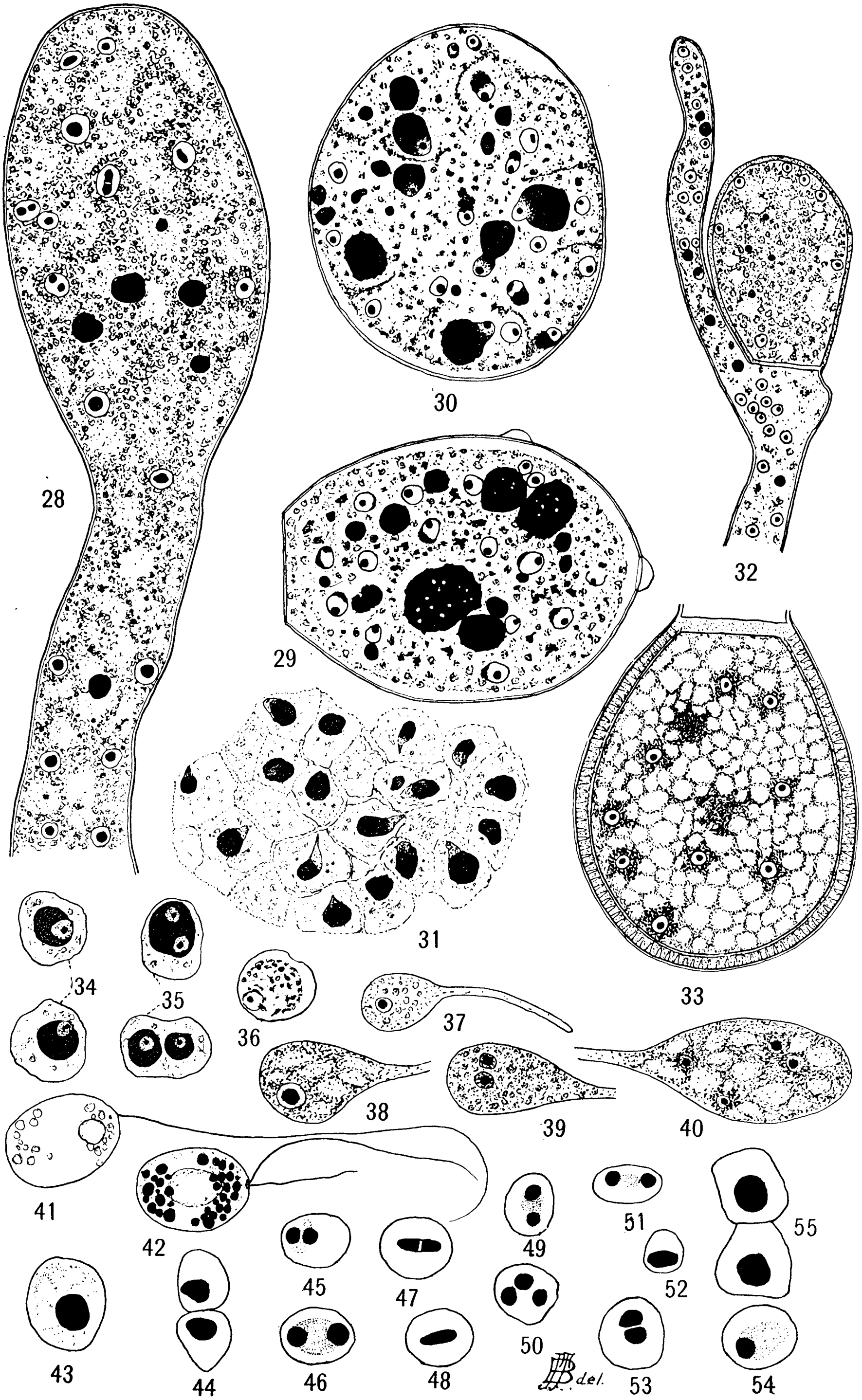
FIG. 31.—Section of a zoosporangium in which segmentation has taken place.

FIG. 32.—Section of a developing resting sporangium and a sub-branch.

FIG. 33.—Section of a mature resting sporangium.



BARRETT on BLASTOCLADIA



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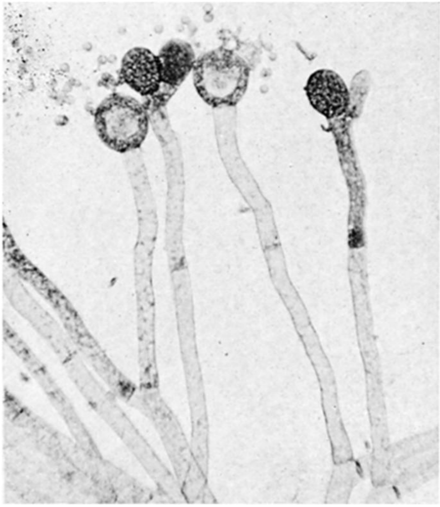

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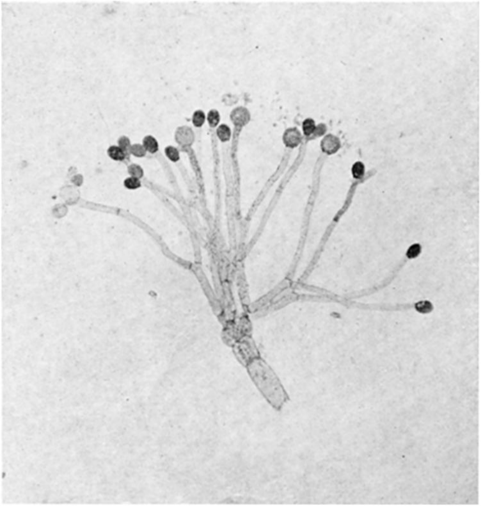

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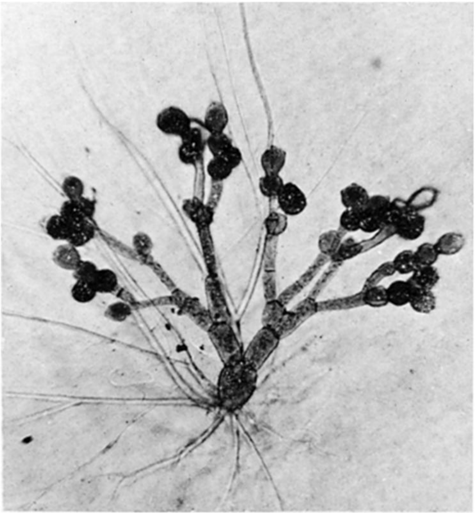

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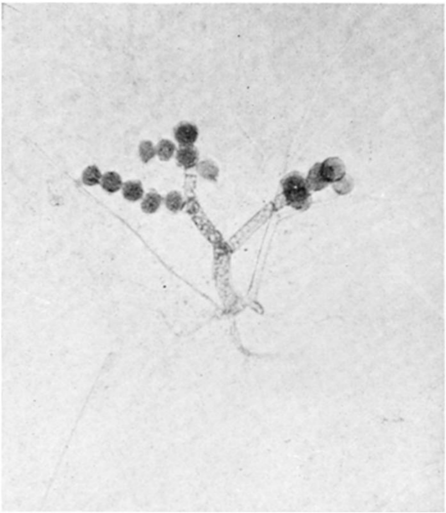

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BARRETT on BLASTOCLADIA

FIG. 34.—Stained zoospore from a ripe zoosporangium: nuclei very distinct; iron-alum hematoxylin.

FIG. 35.—Binucleate zoospores.

FIG. 36.—Zoospore preparing for germination: the large reserve food body is broken up into small granules.

FIG. 37.—Uninucleate germinating zoospores.

FIG. 38.—Germinating zoospore showing the large nucleus just previous to division.

FIG. 39.—Binucleate stage of a germinating zoospore.

FIG. 40.—More advanced condition of a germinating zoospore.

FIG. 41.—Zoospore killed with iodine: nucleus clearly shown, but the food body is invisible.

FIG. 42.—Zoospore killed with weak Flemming's solution: granules surrounding the nucleus and reserve food body stained black.

FIG. 43.—Resting nucleus, showing a large deeply staining chromatin mass surrounded by faintly staining cytoplasm.

FIG. 44.—Late stage in the division of a nucleus.

FIGS. 45–55.—Various stages of nuclear division.

FIG. 56.—A photomicrograph of zoosporangia discharging zoospores, and resting sporangia.

FIG. 57.—A photomicrograph of the plant from which fig. 56 was taken.

FIG. 58.—A photomicrograph of a small entire plant.

FIG. 59.—A photomicrograph of a young plant grown on agar, which shows the arrangement of zoosporangia in chains.